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Conditioned Taste Aversion Induced by Tryptamine: A Temporal Analysis

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FLETCHER, P. J. *Conditioned taste aversion induced by tryptamine: A temporal analysis.* PHARMACOL BIOCHEM BEHAV **25**(5) 995–999, 1986.—The effects of tryptamine on the induction of a conditioned taste aversion (CTA) to a novel saccharin solution were examined. In a single bottle-repeated injection paradigm, tryptamine (40, 60 and 80 mg/kg, IP) induced a significant CTA. This effect was relatively weak, with only the highest dose tested inducing a progressive decline in saccharin intake across trials. The weak action of tryptamine (40 and 60 mg/kg) was confirmed in a more sensitive CTA paradigm which measured the relative preference for saccharin and water. Results of this experiment also showed that prolonging the duration of action of tryptamine failed to enhance the formation of a CTA. This finding extends previous reports that prolonging the duration of action of a compound does not increase potency in the CTA paradigm, and thus casts doubt on the generality of the duration of action hypothesis.

Tryptamine Conditioned taste aversion Duration of action Rat

MANIPULATION of serotonin (5-HT) levels has been shown to alter the behaviour of rats in the conditioned taste aversion (CTA) paradigm. This paradigm involves pairing the ingestion of a novel tasting fluid with a subsequent drug injection. Following recovery, a CTA is said to develop if the animals avoid this flavour.

Various drugs altering 5-HT levels have been reported to induce a CTA. These include fenfluramine [2], fluoxetine [25], 5-hydroxytryptophan (5-HTP) [12, 29, 31], *p*-chloroamphetamine [18] and quipazine [13]. Serotonergic agents induce consistent effects on other behavioural measures. Thus, these drugs reduce food intake ([1] for review). Similarly, at high doses these agents induce a motor syndrome characterized by hyperactivity, forepaw treading, head weaving, hind-limb abduction and Straub tail ([21] for review).

The biogenic trace amine tryptamine may act as a direct 5-HT agonist and a modulator of 5-HT neurotransmission [23]. This amine induces similar behavioural changes to those induced by the serotonergic drugs listed above. Tryptamine reduces food intake [8] and induces the 5-HT syndrome in tranylcypromine treated rats [26]. The effects of tryptamine have not been investigated in the CTA paradigm and so the present experiments were designed to examine the ability of tryptamine to induce a CTA.

EXPERIMENT 1

Method

Thirty male Wistar rats weighing 210–250 g were housed in groups under a 12-hour light/dark cycle with food available at all times except during testing.

Procedure

The rats were water-deprived for 23 hr and then individually given access to a single water bottle in the test cage for two 15 minute periods beginning at 10:00 a.m. and 4:00 p.m. When water intakes had stabilised on this schedule the rats were randomly assigned to one of five groups (n=6), which were then matched for baseline water intake. On the following day all rats were presented with a single bottle of 0.1% sodium saccharin (Sigma Chemical Co., St. Louis, MO) during the morning access period. Immediately afterwards, each group was injected as follows: group 1—saline; groups 2–5—20, 40, 60 and 80 mg/kg tryptamine hydrochloride (Sigma Chemical Co., St. Louis, MO). Water was available as usual that afternoon and for the following 3 days. The animals were then presented with 0.1% saccharin in the morning and injected immediately afterwards as before. This day, therefore, represented both a retention trial to examine the effects of the first saccharin-drug pairing and as a second conditioning trial. Water was available as usual in the afternoon. This cycle of morning access to saccharin followed by three days of access to water only was repeated four times. No drugs were injected after the fourth exposure to saccharin.

Tryptamine hydrochloride was dissolved in 0.9% saline and injected in a volume of 1 ml/kg (IP). The fluid consumption of each animal was measured to the nearest 0.1 ml. The doses of tryptamine were chosen on the basis of previous behavioural experiments. Tryptamine (40–80 mg/kg) reduces food intake by more than 60% [8], and in the dose range 20–80 mg/kg suppresses responding for electrical stimulation of the median raphé nucleus [3].

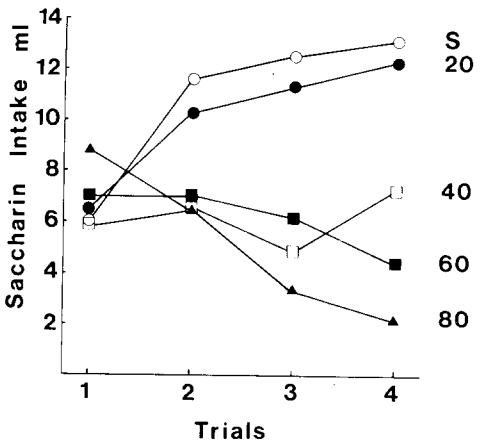

FIG. 1. The effect of repeated pairings of various doses of tryptamine and saline with saccharin intake. Trial 1—saccharin intake prior to injection; trials 2, 3 and 4—saccharin intake on each retention trial; trials 1, 2 and 3 served as conditioning trials. Each point represents the mean saccharin intake for 6 rats. Standard errors are omitted for clarity (see text for statistical details).

Results and Discussion

The mean saccharin intakes of each of the five groups are illustrated in Fig. 1. Two-way analysis of variance using groups and trials as factors, with repeated measures on the trials factor showed a significant main effect of groups, $F(4,25)=6.84$, $p<0.001$. A significant main effect of trials, $F(3,75)=3.81$, $p<0.05$, and a significant groups × trials interaction, $F(12,75)=13.24$, $p<0.01$, reflected the fact that rats treated with saline or 20 mg/kg tryptamine tended to increase consumption over trials, whereas rats treated with 40, 60 or 80 mg/kg tryptamine maintained, or decreased intake over trials. Multiple comparisons (Dunnett $\alpha=0.05$) showed that 40, 60 and 80 mg/kg tryptamine significantly reduced saccharin consumption relative to controls on each retention trial.

Thus, following repeated pairings of saccharin with 40, 60 or 80 mg/kg tryptamine, significant CTAs were observed. Examination of Fig. 1 shows that the mean saccharin intakes for the groups treated with 40 and 60 mg/kg tryptamine remained consistent across trials, and did not appear to differ from the saccharin intake on the first conditioning day, before the rats had experienced tryptamine. Only the group treated with 80 mg/kg tryptamine showed a progressive decline in saccharin intake across trials which is characteristic of a strong stimulus for CTA acquisition in this paradigm. Thus, at doses which are behaviourally active [3,8] tryptamine is a relatively weak stimulus for the formation of a CTA.

Two explanations which can be offered to account for the weak action of tryptamine are concerned with the paradigm used to measure the CTA, and the pharmacological properties of tryptamine. The procedure used in this experiment is less sensitive than the two bottle preference test in which animals are allowed to choose between ingesting the target flavour and water [22]. The following experiment further examined the effects of tryptamine on the acquisition of a CTA using this paradigm. It has been proposed that the duration of action of a compound may be an important factor in determining potency in CTA learning (e.g., [4, 5, 7, 19]). Since tryptamine is rapidly catabolised in rat brain [10,30], it is possible that this short half-life is responsible for the weak action of tryptamine. This issue was also examined in the following experiment by investigating the effects of tryptamine on the acquisition of a CTA using a procedure designed to prolong the duration of action of tryptamine.

EXPERIMENT 2

Previous studies investigating the duration of action hypothesis have compared the effects of a single drug dose on the induction of a CTA against the effects of multiple, divided doses of the drug [6, 7, 15]. Although this procedure ensures that all subjects are injected with the same total amount of drug, the peak effect of the drug is reduced in animals which receive the drug in divided doses [6]. In order to overcome this problem, the following experiment compared the effects of a single dose of tryptamine with the effects of three spaced injections of the same dose of tryptamine. Following intraperitoneal injection tryptamine is almost completely eliminated from the body within 30 minutes [11]. In this experiment, therefore, tryptamine injections were spaced at 20 minute intervals. This procedure was adopted to preserve the peak effect of the tryptamine doses used, and as an attempt to prolong the pharmacological activity of tryptamine.

Method

Thirty male Wistar rats weighing 210–250 g at the time of testing were housed in groups under the condition described in Experiment 1.

Procedure

The rats were water-deprived for 23 hr and then individually given access to two water bottles in test cages for a 30 minute period each day. Once water intake had stabilised on this schedule, the rats were assigned to one of five groups (n=6) which were matched for daily water intake. On the following day all rats were allowed access to two bottles containing a solution of 0.1% sodium saccharin for 30 minutes. Immediately afterwards each rat was given three separate injections (IP) spaced 20 minutes apart according to the following groups: group 1—3 saline; group 2—40 mg/kg tryptamine + 2 saline; group 3—3×40 mg/kg tryptamine; group 4—60 mg/kg tryptamine + 2 saline; group 5—3×60 mg/kg tryptamine. The drug injection procedure was as described for Experiment 1. On each of the following four days each animal was simultaneously given access to two bottles, one containing 0.1% saccharin and the other containing water for 30 minutes. The amounts of each fluid consumed were recorded to the nearest 0.1 ml.

Results and Discussion

The saccharin intake for each rat on each of the four extinction trials was converted to a percentage of the total fluid intake to yield a preference score. The mean saccharin preference scores for each group are shown in Fig. 2.

Two-way analysis of variance using groups and trials as factors, with repeated measures on the trial factors, showed only a small significant effect of groups, $F(4,25)=2.82$, $p<0.05$. The effect of trials, $F(3,75)=19.24$, $p<0.001$, was significant but the groups × trials interaction did not reach significance, $F(12,75)=0.99$, $p>0.1$. Multiple comparisons

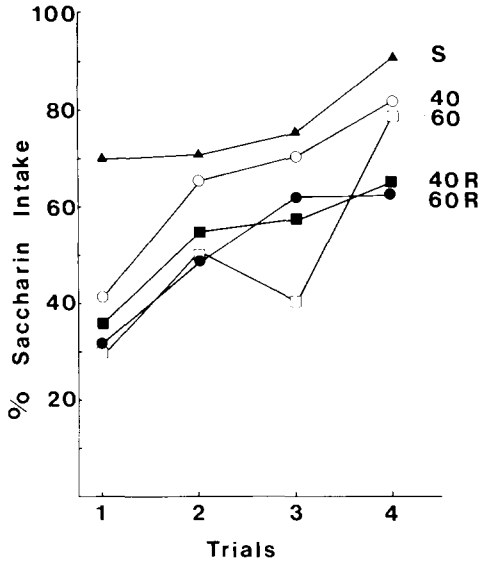

FIG. 2. The effect on subsequent saccharin preference of pairing a single injection of tryptamine (40 and 60 mg/kg), or three repeated (R) injections of tryptamine (40 and 60 mg/kg) with saccharin consumption. Saccharin consumption is expressed as a percentage of total fluid intake on each of four two-bottle choice trials. Each point represents the mean saccharin preference score for 6 rats. Standard errors are omitted for clarity (see text for statistical details).

(Newman-Keuls, $\alpha=0.05$) revealed that all groups treated with tryptamine exhibited reduced saccharin preference compared to controls on trial 1. This effect extinguished on trial 2, although the group treated with a single injection of 60 mg/kg tryptamine showed a significantly reduced preference for saccharin on trial 3. There were no differences between the group receiving a single injection of tryptamine and the group receiving 3 injections of tryptamine at either dose level.

These results confirm the findings of Experiment 1 that doses of tryptamine which reliably alter behaviour [3,8] induce a weak CTA. This weak action of tryptamine does not appear to be the result of the short half-life of the amine since prolonging its duration of action did not facilitate the acquisition of a CTA.

Previous studies have shown that multiple, divided doses of LiCl [7] and divided infusions of cocaine [15] induce an enhanced CTA compared to treatment with a single injection of the same total dose. The present experiment differs from these studies in that some subjects received three times more tryptamine than those receiving a single dose of tryptamine. It is, therefore, significant that three spaced injections of 40 or 60 mg/kg tryptamine did not enhance the CTA induced by a single dose of 40 or 60 mg/kg tryptamine. However, in view of the short half-life of tryptamine [10,30], and the pharmacokinetic properties of intraperitoneally administered tryptamine [11], described above, it is unlikely that these subjects received the functional equivalent of a single injection of 120 or 180 mg/kg tryptamine in terms of rate of onset of action, duration of action and peak effect.

GENERAL DISCUSSION

These experiments show that tryptamine like other 5-HT acting agents induces a CTA. This effect is relatively weak, and is observed only when a high dose of tryptamine is administered. Repeated pairings of saccharin and 40, 60 or 80 mg/kg tryptamine produced statistically reliable aversions to saccharin, but the amounts of saccharin ingested did not decline over trials except for the group treated with the highest dose of tryptamine. This weak action of tryptamine was confirmed using a two-bottle preference test. Following a single pairing of saccharin and 40 or 60 mg/kg tryptamine the subsequent preference for saccharin was reduced only on the first retention trial. This aversion showed rapid extinction. Thus, the weak action of tryptamine was demonstrated in two different CTA paradigms.

Although it has been suggested that a long duration of action is necessary for the acquisition of a drug-induced CTA [4, 5, 7, 19], this does not appear to hold for tryptamine. In Experiment 2 multiple-injections of tryptamine to produce a longer duration of action were no more effective at inducing a CTA than a single dose of tryptamine. The failure of three spaced injections of tryptamine to enhance the acquisition of a CTA cannot be attributed to a floor effect. A single injection of 80 mg/kg tryptamine has been shown to induce a CTA which is resistant to extinction in this two bottle paradigm [11]. The results of Experiment 2 complement previous reports that prolonging the duration of action of apomorphine, intraperitoneal cocaine [6] and β-phenylethylamine [9] did not induce enhanced CTAs to these compounds. A recent study [17] reported that cathinone, a phenylethylamine derivative structurally related to amphetamine, is a weak stimulus for inducing a CTA. A comparison of the potency ratios for cathinone and amphetamine showed that cathinone was 17 times less potent than amphetamine in inducing a CTA, but only 4 times less potent than amphetamine in reducing water intake. Since both compounds had similar time courses of action in the test of adipsia factors related to the duration of action of cathinone cannot account for the low potency of this drug in the CTA paradigm [17].

It is unclear why extending the duration of action of tryptamine, and other compounds [6,9], fails to enhance the formation of a CTA, whilst prolonging the effects of LiCl [7], nitrous oxide [19] and infused cocaine [15] markedly facilitates the development of a CTA. Drug-induced CTA is not a unitary phenomenon involving a single mediating mechanism [16], and it is possible that for certain compounds a long duration of action may be necessary to induce a CTA. However, the present results, and those reported previously [6,9], clearly indicate that a long duration of action is not a sufficient condition for the formation of a CTA. Overall these results cast doubt on the generality of the duration of action hypothesis.

Pharmacokinetic factors other than duration of action may be crucial to the development of a drug-induced CTA, and may be important for understanding why prolonging duration of action does not always facilitate the formation of a CTA. For example, it has been speculated [19,28] that a gradual rate of onset may be required for a drug to induce a CTA. Switzman *et al.* [28] have suggested that the weak CTA induced by heroin may be the result of the rapid entry of this drug into the brain. Similarly the weak CTAs induced by β-phenylethylamine [9], cocaine [5, 6, 15, 20] and 5-HT [14] may be due to the fact that these compounds have a rapid onset of action, as measured by their effects on activity [9], stereotypy [24] and feeding [27], respectively. Thus, the failure to demonstrate enhanced CTA, with β-phen-

ylethylamine [9] and intraperitoneally administered cocaine [6], despite prolonged duration of action may have occurred simply because these compounds have a rapid onset of action. Recently it has been shown [11] that plasma levels of tryptamine are significantly elevated 5 minutes after a single intraperitoneal injection indicating a very rapid onset of action. Therefore, a similar argument could be advanced to account for the weak action of tryptamine, and of repeated injections of tryptamine in the CTA paradigm. However, the idea that drug potency in the CTA paradigm is related to the rate of onset of drug action is speculative and further experiments are necessary to test this hypothesis.

Finally, it is worth considering the possible site of action of the tryptamine-induced CTA. Although tryptamine can cross the blood brain barrier, very little was found in the brain following an intraperitoneal injection of 25 mg/kg tryptamine [11]. This dose of tryptamine is considerably lower than that required to induce a CTA, and so it is unclear whether the CTA results from central or peripheral action. However, systemically administered 5-HT, at doses which do not penetrate the blood drain barrier, induces marked hypophagia and hyperphagia and a comparatively weak CTA [14]. In keeping with this finding it has been reported that the 5-HTP-induced CTA is mediated peripherally [12], although the results of a recent study failed to support this conclusion [29]. Evidence, therefore, suggests a consideration of the possibility that the tryptamine-induced CTA involves a peripheral site of action. Further work is required to determine whether the site of action is related to serotonergic mechanisms.

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